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Biochemical reactions for testing water supplies

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BIOCHEMICAL REACTIONS FOR TESTING WATER SUPPLIES

BY

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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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ENTITLED Biochemical Reactions for Testing Water Supplies.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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An abundance of wholesome water has always been and always will be of vital importance to a municipality. Our largest cities in America, for example, New York, Chicago, and Philadelphia, are spending millions of dollars on water works systems with the expectation of supplying their citizens with pure water. New York, for instance, is constructing an aqueduct about ninety miles in length to convey the water collected on the uncontaminated watersheds in the Catskill Mountains to the Metropolis. Chicago has improved, and is still further improving its water supply by building drainage canals to dispose of its sewage, so as to keep Lake Michigan free from contamination. Experiments are also being carried on by the Sanitary District of Chicago to determine the most suitable means of purifying the sewage of the city of Chicago. Philadelphia and many other cities are purifying river water by filtration.

Whichever method is followed, there must be a suitable control in order to keep the quality of the water above suspicion. The kind of control needed is determined by systematic sanitary examinations of the water. In some cases complete sanitary analyses are not needed. In determining the efficiency of a filter, it is usually only necessary to make a few regular tests.

A complete sanitary analysis of a water* consists of four

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*Whipple, G. C. The Microscopy of Drinking Water p.8

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parts, the physical, the microscopical, the bacteriological, and the chemical. The data obtained are as follows:

PHYSICAL EXAMINATION.

Temperature-Turbidity-Color-Odor, (both cold and hot).

MICROSCOPICAL EXAMINATION.

Number and kind of microscopic organisms per c.c.-

Amount of inorganic matter, amorphous matter, etc.

BACTERIOLOGICAL EXAMINATION.

Number of bacteria per c.c.-Presence of intestinal bacteria or others associated with pollution.

CHEMICAL EXAMINATION.

Total Residue on Evaporation-Loss on Ignition-Fixed Solids-Alkalinity-Hardness-Chlorine-Iron-Nitrogen as Albuminoid Ammonia-Nitrogen as Free Ammonia-Nitrogen as Nitrites-Nitrogen as Nitrates-Total Organic Nitrogen (Kjeldahl Method) - Oxygen consumed-Dissolved Oxygen-Free Carbonic Acid, etc. (Some of these are of use only in special cases)

An analysis of this kind is intended to show whether or not the water would cause sickness if used for drinking; whether or not it contains anything that would render it distasteful or unpalatable; whether or not it contains any ingredient that would make it unfit for laundry use or for general or industrial purposes. The value of the analysis is in its interpretation, each part of the analysis must be interpreted by comparison with all the other parts and in the light of exact knowledge of the environment of the water. In the detection of pollution the chemical and bacteriological examinations furnish the most information, in the study of the aesthetic qualities of a water the physical and microscopical examinations are most important, while in investigations concerning

the value of a water for industrial purposes the chemical and physical examinations may alone suffice.

According to Mason* a thorough study of the source whence a water comes and of the opportunities for pollution, both constant

*Mason, W. P. Examination of Water. p.152

and occasional, to which it may be exposed is termed the "Sanitary Survey". If but one form of examination of a water is possible, the sanitary survey should be the one selected. No amount of inspection could, however, be substituted for the bacterial count in testing the efficiency of a filter plant, nor would it be of value in warding off danger to a ground-water arising from the presence of an unsuspected cesspool.

Of the four parts of a Sanitary analysis the physical examination seems to be the less important.

Whipple* states that the object of the microscopical ex-

*Whipple, G. C. The Microscopy of Drinking Water. p. 10

amination may be considered in five aspects: 1. As indicating sewage contamination. 2. As explaining the chemical analysis. 3. As explaining the cause of turbidity, odors, etc., in water. 4. As a means of identifying the source of a water (in special cases). 5. As a method of studying the food of fishes and other aquatic animals.

1. The microscopical examination cannot be depended upon to determine the pathogenic qualities of a drinking-water, nor can it show definitely whether a water is polluted by sewage unless the pollution is excessive. It can, however, give evidence which, taken with the chemical and bacteriological examinations, may establish

the proof. By revealing microscopic organisms and objects the microscopic examination leads one to suspect the purity of the water.

2. The chemical examination determines the amount of organic matter that a sample of water contains, but it does not determine the nature of it, which is important. The microscopical examination in addition gives valuable information by showing not only whether the organic matter in suspension is vegetable or animal, but by determining whether it is made up of living organisms or of decomposing fragments.

3. By far the most important service that the microscopical examination renders is that of explaining the cause of the taste and odor of a water and of its color, turbidity, and sediment. Several of the microscopic organisms give rise to objectionable odors in water, and, when sufficiently abundant, have a marked influence on its color. They also make the water turbid and cause unsightly scums and sediments to form. Upon all such matters related to the aesthetic qualities of a water the microscopical examination is almost the only means of obtaining reliable information.

4. The presence of certain microscopic organisms in water sometimes gives a clue to its origin. In this way the presence of surface water in a well may be detected. In the Chicago Drainage Canal case the presence of Lake Michigan water in the St. Louis water supply was indicated by finding in it certain diatoms characteristics of the Lake Michigan water.

5. The microscopic organisms form the basis of the food supply of fish and other aquatic animals. The presence or absence of certain fish in a water depends upon the abundance of microscopic life in a water.

Savage* says that the object of the chemical and bacteriol-

*Savage, W. G. The Bacteriological Examination of Water Supplies p.178-180.

ogical examinations of water supplies is to determine not only whether the water is actually polluted with matters of excrementitious and harmful nature, but also whether it is liable to pollution in the future. A water at the time of its examination may be free from actual contamination, and yet be liable to such pollution. The bacteriologist is in a position with a bacteriological examination to say whether a water has been polluted with excrementitious matter or not; and further, to say whether it is, or is not, at the time of examination, showing evidence of continued pollution. On the other hand, from the results of such an examination it is never possible to say that the water will remain free from excretal contamination. The sanitary-chemical and the bacteriological examination of water supplies should never be separated from one another. "In general, the chemical figures show much less fluctuation and variation from season to season, and the method appears to be much less sensitive. A water which is contaminated sufficiently to yield evidence of such pollution by chemical analysis will usually show overwhelming evidence pointing to the same conclusion on bacteriological examination; while many waters, on the other hand, show pollution by bacteriological methods which on chemical analysis alone are above suspicion. The bacteriological data require, however, much greater skill and experience to interpret, while the possibility of false deductions from faulty collection or local contamination is very much greater."

The bacteriological analysis* gives a correct picture of

*Prescott and Winslow. Elements of Water Bacteriology. p. 183.

the hygienic condition of the water at the moment of examination. The evidence furnished by inspection and by chemical analysis should be sought for and welcomed whenever it can be obtained, yet on account of their directness, their delicacy, and their certainty, the bacteriological methods should least of all be omitted, and, if necessary, they alone may furnish conclusive testimony as to the safety of a potable water.

It would seem that bacteriology* deals with the present

*Mason, W. P. Examination of Water. p. 156.

and that chemistry, besides throwing light upon the past, does, to some degree, prophecy what may happen in the future.

The direct chemical analysis*, being the broadest of all,

*Sellards, A. W. J. Infect. Dis., Chicago, 1907, Supple III. p. 42.

has one advantage over all, or one disadvantage, as the case may be. Such an analysis does not depend on the presence of living active organisms as do all bacterial methods; but the pollution may still be detected where bacteria have died in large quantities after exhausting their food material. Also the chemical analysis will detect the pollution where soil-filtered sewage reaches a water supply. Probably the most necessary factor in interpreting the sanitary chemical analysis is a thorough knowledge of the source of the water under examination.

From what has been said it is evident that the chemical and bacteriological examinations are the most important methods and

both should be made to determine the quality of a water.

Sellards* suggests a further line of procedure to the

*A. W. Sellards, J. Infect. Dis., Chicago, 1907 III. p. 43.

scientist, that is, the chemical analysis of bacterial cultures obtained by the inoculation of water samples into artificial media. The principle upon which these experiments were based is on the supposition that, by the inoculation of water into sterile media, we could, in a way, imitate the changes that would ordinarily be brought about by bacteria in a water containing natural media. One great advantage would be in the ease with which the artificial media could be analyzed, and the accuracy with which the changes due to the water could be determined.

According to Sellards* the results of the chemical and

*Sellards, A. W. University of Illinois , Water Survey Series No. 7, p. 40-41.

bacteriological analyses may frequently bear but little direct relation to each other. For example, in a water which is in excellent bacteriological condition, the ammonia content and other constituents may be very high. The reverse situation may also occur and these factors may be comparatively low in waters which are moderately polluted. This lack of correlation can be partly obviated by modification of the current methods of analysis. When water samples are inoculated into nutrient broth, the changes which take place in the media- such as ammonia formation- depend upon the bacterial condition of the inoculated samples and are practically independent of the ammonia content, for example, of the original sample. Hence,

while there may be no relation between the ammonia content and the bacteriological condition of a water sample, yet the ammonia formed in artificial culture was at least approximately proportional to the bacterial condition of the water in question. From a bacteriological standpoint, the central problem in sanitary water analysis is the recognition of the colon group in mixed cultures by rapid methods which give some approximation of the numbers of colon bacilli present.

A series of experiments made by Dr. Sellards* with a Nitrite

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*Sellards, A. W. University of Illinois, Water Survey Series No. 7, p. 42.

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broth is summarized as follows:

The medium used in the nitrite* investigation was an

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*Sellards, A. W. Current Methods of Sanitary Water Analysis, Water Survey Series No. 7, p. 46-60.

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ordinary meat extract broth of double concentration with an acidity of 2% of normal acid, and to this was added 2% of gelatin and 0.05% sodium nitrite. Precautions were taken to adjust the final acidity before adding the sodium nitrite in order that any loss of nitrite during heating and sterilization might be uniform in different lots of media. Five c.c. of the broth were measured accurately into test tubes. Intermittent sterilization was employed. The medium was stored at a temperature of 8° and under these conditions it was solidified. Inoculations were made with 1 c. c. quantities of the water to be tested or where pure cultures were used, the bacteria were inoculated directly without correcting for the volume of 1 c.c. used in the water samples. Incubations were carried on at from 37°

to 39° C for 48 hours, at the end of this time tests for nitrite were made.

Analyses of a polluted creek and a pure well water gave results as follows:

	Nitrogen as Nitrites parts per million.
Pure Well	25
Polluted Creek	0
Sterile Media	25

This experiment was repeated three times with similar results. A series of tests were then made upon pure cultures to determine whether or not the reaction is of differential value. From the results of these tests it appeared that taken as a class, the intestinal bacteria are very active in destroying nitrites. Those bacteria which were most active in forming ammonia as shown in a previous experiment,* did not destroy nitrites in the nitrite broth.

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*Water Survey Series No. 7, University of Illinois Bulletin, p. 45

The nitrite test seems to be very delicate. In the case of four dug wells representing moderate pollution, the time required for the complete destruction of the nitrite varied from eighteen to thirty-six hours. Upon deep driven and carefully protected dug wells the time was much longer, ranging from four days to two weeks. Comparison with the dextrose broth fermentation test was made to determine the delicacy of the reaction. Sterilized water was polluted artificially with pure cultures of B. Coli and with sewage.

Two cultures of coli were used, one of which fermented and one which did not ferment saccharose. Emulsions of the bacteria were prepared in water and successive ten-fold dilutions prepared.

One c.c. quantities of these various dilutions were inoculated into fermentation tubes containing glucose broth and into nitrite broth. The dilution of the emulsions which gave gas in the glucose broth was, in every case, also sufficient to completely destroy all the nitrites in the media within at least forty-eight hours. Tests upon diluted sewages gave less definite results, but where gas production, typical of B. Coli was obtained, the same dilution also gave complete destruction of nitrites within a forty-eight hour limit.

No attempt was made to determine the exact changes which take place during the destruction of the nitrites. It is not improbable that the process is a reduction resulting in the formation of amines, ammonia and elementary nitrogen.

B. Coli reduce nitrates to nitrites so in the presence of this organism no oxidation to nitrates is possible. Bacteria seem to be the cause for the destruction of the nitrites in the media for in one series where an inhibiting agent was used there was no reduction of the nitrite content, whereas, a series without the inhibiting agent gave complete destruction of the nitrites. It is an open question whether the destruction of nitrites results from direct bacterial action or from secondary processes. Media used in the nitrite test must, of course, be stable towards sodium nitrite. The amount of acid used in the medium depends largely upon the quantity of albumen present, for the albuminous-like material binds the acid and protects to a large extent the sodium nitrite being liberated from the solution as nitrous acid.

The nitrite test was made on several (131) regular routine samples received for examination by the State Water Survey, and compared with the dextrose fermentation tests. The results were

as follows:

		No.	Per Cent
Agreements		91	69
Disagreements		19	15
Indeterminate	(Glucose media	21	16
	($NaNO_2$ media	0	0
	Total	131	

By altering the amount of acid, the amount of albuminous material or the quantity of sodium nitrite, the destruction time might be reduced to twenty-four hours.

Tables of the analysis of the routine samples of the State Water Survey are from page 54 to 59 inclusive, Water Survey Series No. 7, University of Illinois.

EXPERIMENTAL PART

The chemical and bacteriological analyses, as performed in the laboratory of the Illinois State Water Survey to determine the purity of a water, take from forty-eight to seventy-two hours. The length of time is determined by the time required by the longest test. Confirmatory tests for the Colon Bacillus require the most time, and any shortening of these tests will be helpful. The presumptive tests for B. Coli in mixed cultures take from twenty-four to forty-eight hours. The nitrite destruction method, which has been described, is proposed as an additional or substitute test for determining the presence of B. Coli or other intestinal bacteria, should shorten the time by at least 24 hours. Intestinal bacteria seem to be very active in destroying nitrites* in the nitrite broth. If the

*Sellard, A. W. Water Survey Series No. 7, University of Illinois Bulletin, p. 48.

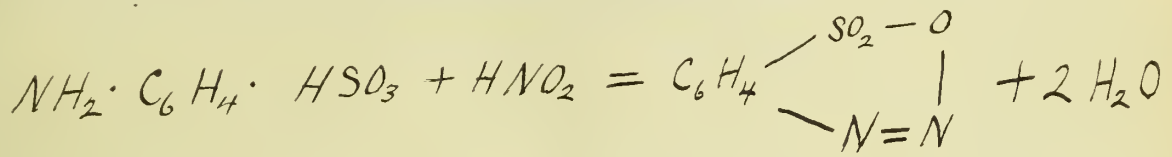
time limit for the destruction of nitrites can be reduced to twenty-four hours, the test will be invaluable. It is simple and would confirm the other presumptive tests for B. Coli.

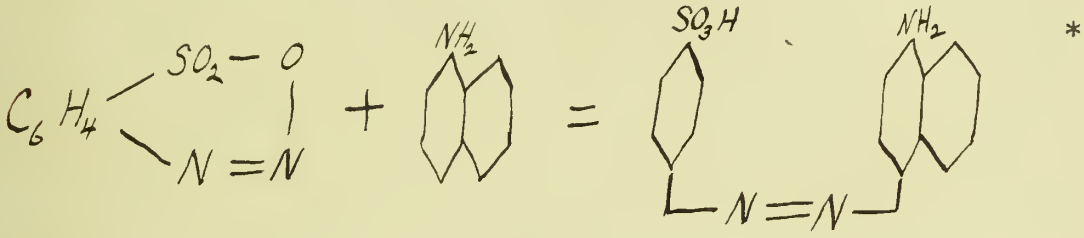
Media was prepared according to the method as directed on page 8. In sterilizing the media, however, ten pounds pressure for 20 minutes in an autoclave was used instead of the intermittent method. One c.c. quantities of the waters were inoculated into the nitrite media. Since Professor Bartow* recommends as a standard for

*Chemical and Biological Survey of the Waters of Illinois, 1908, p62

purity of drinking water that B. Coli should be absent in one c.c., the experiments were carried on using that amount of water. The

nitrite content of the incubated broth was determined by diluting one c.c. with distilled water to 50 c.c., and then adding 1 c.c. sulphanilic acid solution, followed by 1 c.c. of naphthylamine hydrochloride. A red coloration (azobenzolnaphthylamine sulphonic acid) is produced whenever sulphanilic acid and naphthlamine hydrochloride are added to an acidified solution of a nitrite.





*Noyes, W. A. Organic Chemistry 1903. p. 469.

The absence of color in the tests shows that nitrites have been destroyed.

Fifty-seven waters from different sources, but mostly from shallow dug wells, and which were under suspicion were inoculated into the nitrite media. These waters were also tested for gas formers.

The comparative results are as follows, recorded at the end of forty-eight hours:

Result	Method	
	Gas Formers	$NaNO_2$ Media
Positive	44	46
Negative	13	11
Indeterminate	0	0

		Per Cent
Agreements	47	82.45
Disagreements	10	17.54
Indeterminate	0	0
Total	57	

Dr. Sellards obtained 69% agreement on a series of 131 waters, using the same kind of media. (See page 11). The great difference in the percentages is probably due to the class of waters used. Three tests gave positive gas formers in forty-eight hours, but the nitrites were not destroyed until four days after inoculation In three other tests, the nitrite test was positive, while the gas formers were negative in the 1 c.c. sample, yet gas formers were present in 10 c.c. samples of the water. The destruction time in eleven samples was twenty-four hours, and in all of these, gas formers were present in 0.1 c.c. of the water.

In an endeavor to determine the best conditions for the reaction, the acidity of the media which would be most favorable for the destruction of nitrites by intestinal bacteria was next tested. Broths were prepared by dissolving 6 grams of Liebig's Beef Extract, 10 grams of peptone (Wittes) and 20 grams of gelatine in one liter of water. 0.05% of $NaNO_2$ was added just before tubing the media. The broths were marked by A, B, C, D, E, and H, and had a final reaction of neutral, +2%, +4%, +6%, -2%, -4% respectively. Plus (+) signs before percents mean acid, minus signs alkaline, i. e., +2% means two c.c. of normal acid per 100 c.c. of media. These broths were inoculated with waters from different sources, with pure cultures and with a mixture containing all. Table I shows the results. From the results obtained, it is evident that broths A and B give

TABLE I.

NITRITE FERMENTATION TEST

Lab. No.	Source	Gas Formers	SOLUTION A Hours 24	48	72	B Hours 24	48	72	C Hours 24	48	72	D Hours 24	48	72	E Hours 24	48	72	H Hours 24	48	72
20084	River	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+
20085	" Filtered	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20086	Well 36' dug	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20087	Well 160' drilled	-	-	+	+	-	-	+	-	-	-	-	-	-	-	+	+	-	-	-
20088	Well 48' dug	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20089	Well 38' bored	+	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+
20090	Well 32' dug	+	-	-	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-
20091	Well 175' drilled	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20092	Spring	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20093	Well 50' bored	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20094	Well 90' dug-bored	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20095	Well 24' dug	+	-	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
20096	Belleville P. Sta.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20097	Belleville Tap	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20098	Dug Well	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20099	Tap	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20100	Well 20' dug	+	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20101	Dug Well	+	-	+	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-
20102	Well 80' drilled	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20103	80' dug-bored well	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20104	Well 18' dug	+	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-
20105	Well 20' dug	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
I	B. Prodigiosus		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
II	B. Subtilis		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
III	B. Mycoides		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
IV	B. Megatherium		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
V.	B. Coli		+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	-	-	-
VI	B. Vulgaris (Proteus)		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VII	B. Porteus Vulgaris		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
VIII	Mixture of Bacteria		+	+	+	+	+	+	+	+	+	-	+	+	-	-	-	-	+	+

TABLE II NITRITE FERMENTATION TEST

SOLUTION

Lab. No.	Gas Formers	A Hours 24	A Hours 48	A Hours 72	B Hours 24	B Hours 48	B Hours 72
20111	-	-	-	-	-	-	-
20112	-	-	-	-	-	-	-
20113	+	-	+	+	+	+	+
20114	+	-	+	+	-	+	+
20115	+	-	+	+	-	-	+
20116	-	-	-	+	-	-	-
20120	+	+	+	+	+	+	+
20122	+	+	+	+	-	+	+
20123	+	+	+	+	-	-	-
20124	+	-	+	+	-	+	+
20125	+	-	-	-	-	-	-
20126	-	-	-	-	-	-	-
20127	-	-	+	+	-	+	+
20128	-	+	+	+	-	+	+
20129	+	+	+	+	-	+	+
20130	+	-	+	+	-	+	+
20131	+	+	+	+	-	+	+
20132	-	-	+	+	-	+	+
20133	+	-	+	+	-	+	+
20134	-	-	-	-	-	-	-
20135	-	-	-	+	-	-	-
20136	+	+	+	+	-	-	+
20137	-	-	+	+	+	+	+
20138	-	-	-	-	-	-	-
20139	+	-	+	+	-	+	+
20140	+	-	+	+	-	+	+
20141	+	-	+	+	-	+	+
20143	+	-	+	+	-	+	+
20144	-	-	-	-	-	-	-
20145	+	+	+	+	-	+	+
20146	-	-	-	-	-	-	-
20147	+	-	-	-	-	-	-
20148	+	+	+	+	+	+	+
20149	+	-	+	+	-	-	+
20150	+	-	+	+	-	-	+
20151	+	+	+	+	+	+	+
20152	+	+	+	+	+	+	+
20153	+	-	+	+	+	+	+
20154	+	+	+	+	+	+	+
20155	-	-	-	+	-	-	-
20156	+	-	-	-	-	-	-

the best results. A neutral or 2% acid reaction is, therefor, the most favorable reaction for the broths to possess. Further experiments were performed with these broths as shown in Table II.

The results are summarized as follows:

	72 hours	
	Broth A	Broth B
	Neutral	+2% acid
Agreements	31	33
Disagreements	10	8

Forty-one tests appear to be too few to enable us to draw conclusions as to the superiority of the neutral broth over the 2% acid broth. However, we believe that either can be used to advantage.

The composition of the media plays an important part in favoring or inhibiting the growth of certain organisms. For instance large amounts of peptone encourage the growth of many common saphrophytes, as well as the intestinal bacteria, while large amounts of gelatine inhibit the growth of all bacteria when peptone is absent. Tables No. III and IV show the results of the experiments from which we draw the above conclusion.

The media in the experiments recorded in Table III contained 3 grams beef extract and 1, 3, and 5% of peptone per liter. Solutions 0, I, and II had a neutral reaction and contained 1, 3, and 5% of peptone respectively. Solutions III, IV, and V had a +2% reaction and contained 1, 3, and 5% peptone respectively. Even some of the common saphrophytes destroyed nitrites in these media.

Media marked I, II and III in Table IV each contained 3 grams of beef extract and they contained 1, 3, and 5% of gelatin per liter respectively. The reactions of the media were +2% and each contained 0.05% $NaNO_2$. Even B. Coli did not destroy the nitrites in these media.

TABLE III

NITRITE FERMENTATION TEST

Lab. No.	Source	Gas Formers	SOLUTION O			SOLUTION I			SOLUTION II			SOLUTION III			SOLUTION IV			SOLUTION V		
			Hours			Hours			Hours			Hours			Hours			Hours		
			24	48	72	24	48	72	24	48	72	24	48	72	24	48	72	24	48	72
20300	Well 30' dug	+	-	-	-	-	+	+	-	+	-	-	-	-	-	+	+	+	+	+
20301	Well 30' dug	+	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+
20302	Well 25' dug	+	-	+	+	-	+	+	-	+	+	-	+	+	-	+	+	+	+	+
20303	Well 25' dug	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	+	-	+	+
20304	Well 20' dug	+	-	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-	+	+
20305	Well 35' dug	+	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+	+	+	+
20306	Well 25' dug	+	-	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-	+	+
20307	Well 20' dug	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+
20308	Wilmette Tap	+	-	+	+	-	+	+	-	+	+	-	-	-	-	+	+	-	+	+
20309	Wilmette Well	+	-	+	+	+	+	+	-	+	+	-	-	-	-	+	+	-	+	+
I	Mixture of Bacteria		-	-	+	-	-	+	-	+	+	-	-	-	+	+	+	+	+	+
II	B. Prodigiosus		-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
III	B. Subtilis		-	-	-	+	+	+	-	+	+	-	-	-	-	+	+	-	+	+
IV	B. Mycoides		-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-
V	B. Megatherium		-	-	-	-	-	+	-	+	+	-	-	+	-	+	+	-	+	+
VI	B. Coli		-	-	-	-	+	+	-	+	+	-	-	-	+	+	+	+	+	+
VII	B. Vulgaris(Proteus)		-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
VIII	B. Proteus Vulgaris		-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	+	+	+
IX	B. Cloaca		-	-	-	-	-	+	-	-	-	-	-	-	-	+	+	-	+	+
X	Sewage Streptococci		-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	+

NITRITE FERMENTATION TEST

TABLE IV

No.	Source	SOLUTION I Hours 24	48	72	120	SOLUTION II Hours 24	48	72	120	SOLUTION III Hours 24	48	72	120
I	Mixture	-	-	-	-	-	-	-	-	-	-	-	-
II	B. Prodigiosus	-	-	-	-	-	-	-	-	-	-	-	-
III	B. Subtilis	-	-	-	-	-	-	-	-	-	-	-	-
IV	B. Mycoides	-	-	-	-	-	-	-	-	-	-	-	-
V	B. Megatherium	-	-	-	-	-	-	-	-	-	-	-	-
VI	B. Coli	-	-	-	-	-	-	-	-	-	-	-	-
VII	B. Vulgaris(Proteus)	-	-	-	-	-	-	-	-	-	-	-	-
VIII	B. Proteus(Vulgaris)	-	-	-	-	-	-	-	-	-	-	-	-
IX	B. Cloaca	-	-	-	-	-	-	-	-	-	-	-	-
X	Sewage Streptococci	-	-	-	-	-	-	-	-	-	-	-	-

NITRITE FERMENTATION TEST

TABLE V

B. Coli Emulsion c. c.	Gas Formers	0.2% Gelatin broth Hours 24	48	72	2% Gelatin broth Hours 24	48	72
1.0	+	+	+	+	+	+	+
0.1	+	+	+	+	-	+	+
0.01	+	+	+	+	-	+	+
0.001	+	+	+	+	-	+	+
0.0001	+	+	+	+	-	-	+
0.00001	+	+	+	+	-	-	+
0.000001	+	+	+	+	-	-	+
Bone Yard c. c.							
1.0	+	+	+	+	+	+	+
0.1	+	+	+	+	-	-	+
0.01	+	+	+	+	-	-	+
0.001	-	-	+	+	-	-	-
0.0001	-	-	-	-	-	-	-
0.00001	-	-	-	-	-	-	-
0.000001	-	-	-	-	-	-	-

Apparently gelatin might be used to advantage as an inhibiting agent,while peptone acts in the opposite direction. There were considerable growths in all the broths whose action is shown in Table III, and practically no growths were visible in the broths whose action is shown in Table IV.

Because of the above results, media were prepared containing a smaller quantity of gelatin. The amount of peptone was thought to be sufficient in quantity and was not changed. The composition of the medium was as follows: six grams of beef extract per liter, 0.2% gelatin, 2% peptone, 0.05% sodium nitrite, with a final reaction of +2% acid. The medium was filtered and tubed as directed on page 8. B. Coli emulsion was made and tenfold dilutions prepared. A sample from the Bone Yard(a polluted stream), was also diluted in the same manner. Comparative tests were made with these dilutions using the 0.2% gelatin broth and the 2% broth described on page 8. The results are shown on Table V.

The dilutions of the B. Coli emulsion were apparently not carried far enough, for gas formers were present in the highest dilution. A destruction of nitrite was obtained in twenty-four hours in the 0.2% gelatin broth with both the dilutions of B. Coli emulsion and Bone Yard, while the destruction time for the 2% gelatin broth was forty-eight hours.

The experiment was repeated twice with variable results, as is shown in tables VI and VII. In table VI there is shown an agreement between the gas formers and the 2% gelatin broth in 48 hours when B. Coli dilutions were used. There was disagreement, however, in the dilutions of water from the Bone Yard. The 0.2% gelatin broth, however, showed agreement between the gas formers

TABLE VI

NITRITE FERMENTATION TEST

B. Coli Emulsion c. c.	Gas Formers	0.2% Gelatin broth Hours 24	48	72	2% Gelatin broth Hours 24	48	72
0.1	+	+	+	+	-	+	+
0.01	+	+	+	+	-	+	+
0.001	+	+	+	+	-	+	+
0.0001	-	+	+	+	-	-	-
0.00001	-	+	+	+	-	-	-
0.000001	-	+	+	+	-	-	-
0.0000001	-	-	-	-	-	-	-
0.00000001	-	-	-	-	-	-	-
0.000000001	-	-	-	-	-	-	-
Bone Yard c. c.							
0.1	+	+	+	+	-	-	-
0.01	-	-	-	-	-	-	-
0.001	-	-	-	-	-	-	-
0.0001	-	-	-	-	-	-	-
0.00001	-	-	-	-	-	-	-

Incubation 38-39°

TABLE VII

NITRITE FERMENTATION TEST

B. Coli Emulsion c. c.	Gas Formers	0.2% Gelatin broth Hours 24	48	72	2% Gelatin broth Hours 24	48	72
0.1	+	+	+	+	-	+	+
0.01	+	-	+	+	-	-	+
0.001	+	-	+	+	-	-	+
0.0001	+	-	+	+	-	-	+
0.00001	-	-	-	-	-	-	-
0.000001	-	-	-	-	-	-	-
0.0000001	-	-	-	-	-	-	-
0.00000001	-	-	-	-	-	-	-
0.000000001	-	-	-	-	-	-	-
Bone Yard c. c.							
0.1	+	-	+	+	-	+	+
0.01	-	-	-	-	-	-	-
0.001	-	-	-	-	-	-	-
0.0001	-	-	-	-	-	-	-
0.00001	-	-	-	-	-	-	-

Incubation 35-39°

and nitrite removal in the tests of dilutions of water from the Bone Yard, but did not show agreements with the dilutions of B. Coli. These comparative tests gave us the impression that the 0.2% gelatin broth was not only more delicate than the 2% gelatin broth, but more delicate than the dextrosebroth test, which was used in determining the presence of gas formers. A repetition of this experiment was made, as shown in Table VII. The results differ from those shown on Table VI in that the nitrites did not disappear so quickly. The reason for this is attributed to the difference in the incubation temperature. The latter was incubated at 35-37°C, while the former at 38-39°C. The temperature of the incubator was brought to 38-39°C. A series of tests were made using 0.2% gelatin broth to obtain, if possible, a check on the results with the broth of the composition shown in Table VI. The results of this experiment are shown in Table VIII. The higher dilutions of B. Coli, instead of giving positive results in 24 hours to correspond with the gas formers, took forty-eight hours for the destruction of nitrite in the broth.

To try to reduce the time required for the nitrite removal, two batches of broth were made containing 0.2% gelatin, 2% peptone, 6 grams of beef extract per liter, and a final reaction of +1.5%. To one of the broths there was added 0.05% sodium nitrite, and to the other 0.02% sodium nitrite. These broths were inoculated with dilutions of B. Coli and water from the Bone Yard. The results of the experiment and of a repetition were not satisfactory. The fault seemed to lie in the composition of the media. We do not think that the change in the reaction of the nitrite content, or the change from +2% to +1.5% was the cause, yet there must have been a mistake somewhere for the results should have at least checked the

TABLE VIII

NITRITE FERMENTATION TEST

0.2% Gelatin Broth

B. Coli Emulsion c. c.	Gas Formers	Hours 24	48	72
0.1	+	+	+	+
0.01	+	+	+	+
0.001	+	+	+	+
0.0001	+	-	+	+
0.00001	+	-	+	+
0.000001	-	-	-	-
0.0000001	-	-	-	-
0.00000001	-	-	-	-
0.000000001	-	-	-	-
Bone Yard				
0.1	+	+	+	+
0.01	+	-	-	-
0.001	-	-	-	-
0.0001	-	-	-	-

results shown in Table VIII. The temperature of the incubations was from 38°-40°C. Better results were obtained as is shown later in Table X.

A medium with a lower nitrite content was inoculated with pure cultures. The composition of the medium was as follows; 6 grams of beef extract per liter, 2% peptone, 0.2% gelatin, 0.005% sodium nitrite, and a final reaction of +2%. The results are shown in Table IX. The only bacteria used which destroy nitrites are B. Coli and B. Cloaca. Two cultures of B. Coli from different laboratories were used in this series of tests. No effort was made to differentiate the characteristics of these organisms. B. Cloaca was not as active as B. Coli in destroying nitrites in the medium as will be observed in the first Series, Table IX. Destruction of nitrite occurred in 24 hours.

Dilutions of B. Coli emulsions and Bone Yard were next prepared and inoculated in the medium. The results are recorded

TABLE IX

NITRITE FERMENTATION TEST

No. 1st. Series	Fermentation Glucose	Destruction of Nitrite Hours 24	48	72
I Proteus Vulgarus	12%	-	-	-
II B. Coli	40%	+	+	+
IIIMyccides(Bacterium)	-	-	-	-
IV Megatherium	-	-	-	-
V B. Cloaca	97%	-	+	+
VI Prodigiosus	-	-	-	-
VIISewage Streptoccocci	-	-	-	-
VIIIB. Subtilis	-	-	-	-
IX B. Coli	30%	+	+	+
2nd Series				
I Proteus Vulgarus	10%	-	-	-
II B. Coli	35%	+	+	+
IIIMycoides(Bacterium)	-	-	-	-
IV Megatherium	-	-	-	-
V B. Cloaca	85%	+	+	+
VI Prodigiosus	-	-	-	-
VIISewage Strpptococci	-	-	-	-
VIIIB. Subtilis	-	-	-	-
IX B. Coli	40%	+	+	+

in Table X. The destruction of nitrite is accomplished in 24 hours in those samples in which gas formers were present. Destruction of nitrite was complete in 48 hours in the 0.01 Bone Yard sample, but no gas formers were present. It was also noticed that this sample had a thick growth. It is possible that gas formers were present in very small numbers and that they were over-grown by other bacteria present, and that the nitrite medium was more favorable to the gas formers, than to the other bacteria present.

This medium was next used in testing several waters, some of good and others of poor character. The results are shown in Table XI. Positive tests for the nitrite destruction which were obtained in 24 hours agreed with the gas formers. Samples 20,998, 20,999, and 21,000 gave positive nitrite test in 48 hours.

TABLE X

NITRITE FERMENTATION TEST

B. Coli Emulsion c. c.	Gas Formers	SOLUTION Hours 24	48	72
.1	+	+	+	+
.01	+	+	+	+
.001	+	+	+	+
.0001	+	+	+	+
.00001	+	+	+	+
.000001	+	+	+	+
.0000001	-	-	-	-
Bone Yard				
.1	+	+	+	+
.01	-	-	+	+
.001	-	-	-	-
.0001	-	-	-	-

NITRITE FERMENTATION TEST

Lab. No.	Gas Formers	Hours 24	48	72	168
20990	-	-	-	-	-
20991	-	-	-	-	-
20992	+	+	+	+	+
20993	-	-	-	-	-
20994	-	-	-	-	-
20995	-	-	-	-	-
20996	-	-	-	-	-
20997	-	-	-	-	-
20998	-	-	+	+	+
20999	-	-	+	+	+
21000	-	-	+	+	+
21001	-	-	-	-	-
21002	-	-	-	-	-
21003	-	-	-	-	-
21004	+	+	+	+	+
21005	-	-	-	-	-
21006	-	-	-	-	-
21007	+	+	+	+	+
21008	+	+	+	+	+

INDOL REACTION.

When cultures of B. Coli either pure or mixed are inoculated in a peptone solution and incubated for some time indol (C_8H_7N) is produced. Indol is an aromatic compound and produces a rose-red color of nitroso-indol when acted upon by nitrous acid. The test is carried out in the laboratory of the State Water Survey as follows: 10c.c. of the water to be tested is added to 10 c.c. of a 1% sterilized peptone solution and the mixture incubated at 40°C for 3 days, after which the mixture is tested for indol.

The method of testing for indol appears to be of great importance. *Mason recommends the addition of 2c.c. of strong sul-

*Mason, W. P. Examination of Water, 4th Edition. p.142.

phuric acid and 2c.c. of a sodium nitrite solution to a 100 c.c. "Nessler" tube, dilute with 50c.c. water, cool, and then pour in the previously cooled incubated culture. A red coloration, forming within half an hour, indicates indol.

Prescott and Winslow* recommend that 1 c.c. of a 0.02% sodium nitrite solution and 1 c.c. of a 1 to 1 solution of sulphuric

*Prescott-Winslow, Elements of Water Bacteriology p.108

acid be added directly to the incubated culture. They recommend that both the incubated culture and the reagents should be cooled on ice before mixing, and the result recorded after an hour of standing.

A very delicate test for indol is described by Grubbs and Francis* as follows: about 10 drops of concentrated sulphuric acid

*Grubbs and Francis, Laboratory Technique, Bul. No.7 of the Hygienic Laboratory, Washington, D. C., p.3

is added to the incubated tube containing the culture, and then shaken; 4 c.c. of a 1-1000 sodium nitrite solution is then run carefully down the side of the tube so as to form a sharp line of contact between the heavier culture and acid, and the lighter nitrite solution. If indol is present, a pink color will contrast sharply with the portions above and below.

This method of Grubbs and Francis has been used in routine water analysis in the laboratory of the State Water Survey for the past year with satisfactory results.

Most of our modern texts on Water Analysis i.e. Mason's Examination of Water; Prescott and Winslow's Elements of Water Bacteriology; Standard Methods of Water Analysis and several others, recommend four days as the incubation time. This time is too long to be used in Commercial water analysis, as time is an important element. Clients are anxious to know the results and steps must sometimes be taken to condemn a supply. At present the incubation time in our laboratory is 72 hours and our results have been very satisfactory since the ring test for indol was adopted.

In an endeavor to shorten the incubation time from 72 hours to 48 hours, experiments were carried on with a 5% peptone solution. The 5% peptone solution was prepared by distributing the required amount of peptone equally throughout the solution and tubing without filtering. Sterilization was accomplished by giving the medium 15 pounds pressure in an autoclave for 15 minutes. A white sediment of peptone remained undissolved in the tubes.

TABLE XII

INDOL PRODUCTION

Lab. No.	Gas Formers in 10c.c.	1.0c.c.	0.10c.c.	1% Peptone Hours 48	72	5% Peptone Hours 48	72
18355	-	-	-	-	-	-	+
18356	+	+	+	-	+	+	+
18357	?	+	+	-	+	+	+
18358	?	+	-	-	-	-	+
18359	+	+	-	-	-	-	-
18360	?	?	+	-	+	+	+
18361	-	-	-	-	-	-	-
18362	-	-	-	-	+	+	+
18363	+	+	+	-	+	+	+
18364	?	?	+	+	+	+	+
18365	+	-	-	-	-	-	+
18366	-	-	-	-	-	-	-
18367	+	+	-	+	+	+	+
18368	+	+	+	+	+	+	+
18369	+	+	+	+	+	+	+
18371	+	+	+	+	+	+	+
18372	+	+	+	-	-	-	+
18373	?	+	-	-	-	-	-
18374	-	-	-	-	-	-	-
18375	?	+	+	-	+	+	+
18376	+	+	+	-	+	+	+
18386	+	+	+	+	+	+	+
18387	-	-	-	-	-	-	-
18388	+	?	-	+	+	+	+
18389	+	?	?	-	-	-	-
18390	+	+	-	-	+	+	+
18391	?	-	-	-	-	-	-
18399	-	-	-	-	+	-	+
18400	+	+	-	+	+	+	+
18401	+	+	-	+	+	+	+
18402	-	-	-	-	-	-	-
18403	-	-	-	-	-	-	-
18405	+	+	-	+	+	+	+
18406	+	+	-	+	+	+	+
18407	+	+	?	+	+	+	+
18408	?	?	?	+	+	+	+
18409	+	-	-	+	+	+	+
18410	-	-	-	-	-	+	+
18411	+	-	-	+	+	+	+
18412	-	-	-	-	-	-	-
18413	-	-	-	+	+	+	+
18414	+	+	+	+	+	+	+
18415	-	-	-	-	-	-	-
18416	+	?	-	+	+	+	+
18417	?	?	?	-	+	+	+
18418	?	?	?	-	-	-	+
18419	-	-	-	+	+	+	+
18420	-	-	-	+	+	+	+

TABLE XIII

Lab. No.	Gas Formers In 10c. c.	1.0c. c.	0.10 c. c.	INDOL PRODUCTION 1% Peptone Hours 48	72	5% Peptone Hours 48	72
18421	-	-	-	+	+	+	+
18422	-	-	-	+	+	+	+
18424	+	+	+	+	+	+	+
18425	+	+	+	+	+	+	+
18426	+	-	-	+	+	+	+
18427	+	?	-	+	+	+	+
18428	-	-	-	+	+	+	+
18458	-	-	-	-	+	-	+
18459	-	-	-	-	-	-	-
18460	+	+	+	+	+	+	+
18462	-	-	-	-	-	-	-
18463	-	-	-	-	-	-	-
18464	+	+	?	-	+	+	+
18465	?	+	?	-	+	+	+
18466	+	?	-	-	+	+	+
18467	-	?	-	-	+	+	+
18468	+	+	-	-	+	+	+
18469	-	-	-	-	-	-	-
18470	+	?	-	-	+	+	+
18471	?	+	?	-	+	+	+
18472	+	+	-	-	+	+	+
18473	+	+	+	+	+	+	+
18474	+	+	-	-	-	+	+
18475	?	+	?	-	+	+	+
18476	?	?	+	+	+	+	+
20880	-	-	-	-	-	-	-
20881	-	-	-	-	-	-	+
20882	+	+	+	+	+	+	+
20883	-	-	-	-	-	-	+
20884	-	-	-	-	-	-	+
20885	?	-	-	-	+	+	+
20886	+	+	-	+	+	+	+
20887	+	+	-	-	+	-	+
20888	+	+	?	+	+	+	+
20889	+	+	+	-	+	-	+
20890	+	+	-	+	+	+	+
20891	+	-	-	-	+	+	+
20892	-	-	-	-	+	+	+
20893	-	-	-	-	-	-	+
20894	+	+	+	+	+	+	+
20908	+	+	-	+	+	+	+
20909	+	+	-	+	+	+	+
20910	+	+	-	-	+	-	+
20911	-	-	-	-	-	-	-
20912	-	-	-	-	-	-	-
20914	+	+	+	+	+	+	+
20915	-	-	-	-	+	-	+
20916	-	-	-	-	+	-	+

TABLE XIV

INDOL PRODUCTION

B. Coli Emulsion c. c.	Gas Formers in	1% Peptune Hours 24	48	72	5% Peptone Hours 24	48	72
.001	+	-	+	+	+	+	+
.0001	+	-	+	+	-	+	+
.00001	-	-	+	+	-	+	+
.000001	-	-	-	-	-	-	-
.0000001	-	-	-	-	-	-	-
Bone Yard c. c.							
.1	+	-	-	+	-	+	+
.01	-	-	-	-	-	-	-
.001	-	-	-	-	-	-	-
.0001	-	-	-	-	-	-	-
.00001	-	-	-	-	-	-	-

Comparisons were made with a 1% peptone solution. Tests for indol were made by the "ring-test", as described above at the end of 48 and 72 hours. Table XII and XIII show that in every instance but seven out of 59, when indol was positive in the 1% peptone solution in 72 hours, positive results with the 5% peptone solution were obtained in 48 hours. In no case was there a positive test in the 1% peptone solution and a negative in the 5% solution in 72 hours. The 5% peptone solution gave a few positive tests where gas formers were absent. In basing an opinion on a bacteriological analysis, no stress is, however, laid upon a positive indol test unless gas formers are present. Positive results were obtained for both the 1% and 5% peptone solutions when pure cultures were used(see table XIV) in 48 hours. In mixed culture, i.e. the 0.1 c.c. Bone Yard, the 5% peptone solution alone gave a positive test in 48 hours.

From these experiments it would appear to us that the 5% peptone solution could be used with advantage for testing for indol in 48 hours in water supplies.

CONCLUSIONS

1. The reaction of the media which appears to be most suitable to gas formers, and which has given satisfactory results in the removal of nitrites, is +2% acid.

2. Peptone can be used to advantage in increasing the nitrite destroying power of bacteria while gelatin can be used for the opposite effect.

3. The time required for the nitrite fermentation test is 24 hours when the medium described on Page 23 is used.

4. The ring test for indol is simple, clear and delicate and has been used in the laboratory of the State Water Survey with satisfactory results.

5. A 5% peptone solution reduces the time required for indol production in polluted waters to 48 hours.

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